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Effects of Cyclic Adenosine 3',5'- Monophosphate on Photoreceptor Disc Shedding and Retinomotor Movement

Inhibition of Rod Shedding and Stimulation of Cone Elongation

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ABSTRACT As a test of the hypothesis that cyclic nucleotides play a role in the regulation of retinomotor movements and disc shedding in the photoreceptor-pigment epithelial complex, we have used an in vitro eyecup preparation that sustains both disc shedding and cone retinomotor movement. Eyecups were prepared in white light from animals in which both shedding and cone movement had been blocked by 4 d of constant-light treatment. In eyecups incubated for 3 h in light, disc shedding was negligible and cones remained in the light-adapted (contracted) position. In eyecups incubated in darkness, however, a massive shedding response (dominated by rod photoreceptors) was induced, and at the same time cone photoreceptors elongated to their dark-adapted position. In eyecups incubated in light, dbcAMP promoted cone elongation and thus mimicked darkness; the dbcAMP effect was potentiated by the phosphodiesterase inhibitors papaverine and 3-isobutylmethylxanthine. In eyecups incubated in darkness, on the other hand, both phosphodiesterase inhibitors and dbcAMP reduced the phagosome content of the pigment epithelium. The effects of dbcAMP on cone elongation and rod shedding appear to be specific in that dbcGMP, adenosine, and adenosine 5'-monophosphate had no significant effect. Our results suggest that cAMP plays a role in the regulation of both retinomotor movements and disc shedding.

INTRODUCTION

Cyclic nucleotides are thought to play a fundamental role in photoreceptor metabolism. Rod-dominated retinas contain high levels of cyclic guanosine

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3',5'-monophosphate (cGMP) in darkness, which is localized mainly in photoreceptors (Orr et al., 1976) and is greatly decreased by light in both intact retinas (Goridis et al., 1977; Kilbride and Ebrey, 1979) and isolated outer segments (Fletcher and Chader, 1976; Woodruff et al., 1977). In contrast, cyclic adenosine 3',5'-monophosphate (cAMP) is more uniformly distributed throughout the retina and exhibits smaller levels of reduction on exposure to light (Orr et al., 1976). Cone-dominated retinas contain more moderate levels of cGMP in darkness, and significant effects of light have not been detected (DeVries et al., 1979; Farber et al., 1981). Results on cAMP in ground squirrel retinas differ in that one group has reported low levels that are relatively unaffected by light (DeVries et al., 1979), whereas another group has reported high levels that are reduced by light (Farber et al., 1981). Much recent work has focused on the rapid hydrolysis of cGMP in isolated rod outer segments or broken membranes by light-activated phosphodiesterase (Fletcher and Chader, 1976; Yee and Liebman, 1978; Wheeler and Bitensky, 1977; Fung et al., 1981), and available data raise the possibility that cGMP hydrolysis may be a fundamental event in visual transduction (Hubbell and Bownds, 1979). A similar role for cAMP in cone-dominated retinas has been suggested (Farber et al., 1981).

Despite a potential role in transduction, light or dark alteration of cyclic nucleotide levels may modulate other light and dark processes within photoreceptors. For example, in frogs, rod disc shedding (Hollyfield et al., 1976; Basinger et al., 1976) and disc assembly (Besharse et al., 1977) are stimulated by light, and in the case of shedding, prior dark treatment is required (Currie et al., 1978). Light and dark processes also regulate retinomotor movements in lower vertebrates (Arey, 1915). Cone photoreceptors elongate in darkness and contract in light, whereas rods exhibit opposite movements (Arey, 1915; Levinson and Burnside, 1981). Retinomotor movement and shedding occur slowly, taking 1–2 h for the full light or dark response and both phenomena are also influenced by an endogenous circadian rhythm (Besharse et al., 1977; Levinson and Burnside, 1981). Recent studies indicate that retinomotor movements in fishes are influenced by cAMP (Burnside et al., 1982) and that cAMP also inhibits phagocytosis of isolated outer segments when added to monolayer cultures of pigment epithelial cells (Edwards and Bakshian, 1980). These effects of cAMP prompted our investigation of the role of cAMP in the control of retinomotor movement of cones and disc shedding in eyecups prepared from *Xenopus laevis*.

We have recently developed an in vitro preparation that sustains both rod disc shedding (Besharse et al., 1980) and, as we report here, cone retinomotor movements as well. This culture system is particularly useful because it allows experimental manipulation while maintaining close anatomical apposition of photoreceptors and pigment epithelium. In addition, the responses studied in this system correspond closely to those seen in intact animals. Our in vitro preparation has made it possible to test directly the hypothesis that cyclic nucleotides may affect disc shedding and retinomotor movement. We find that addition of exogenous derivatives of cAMP to medium containing

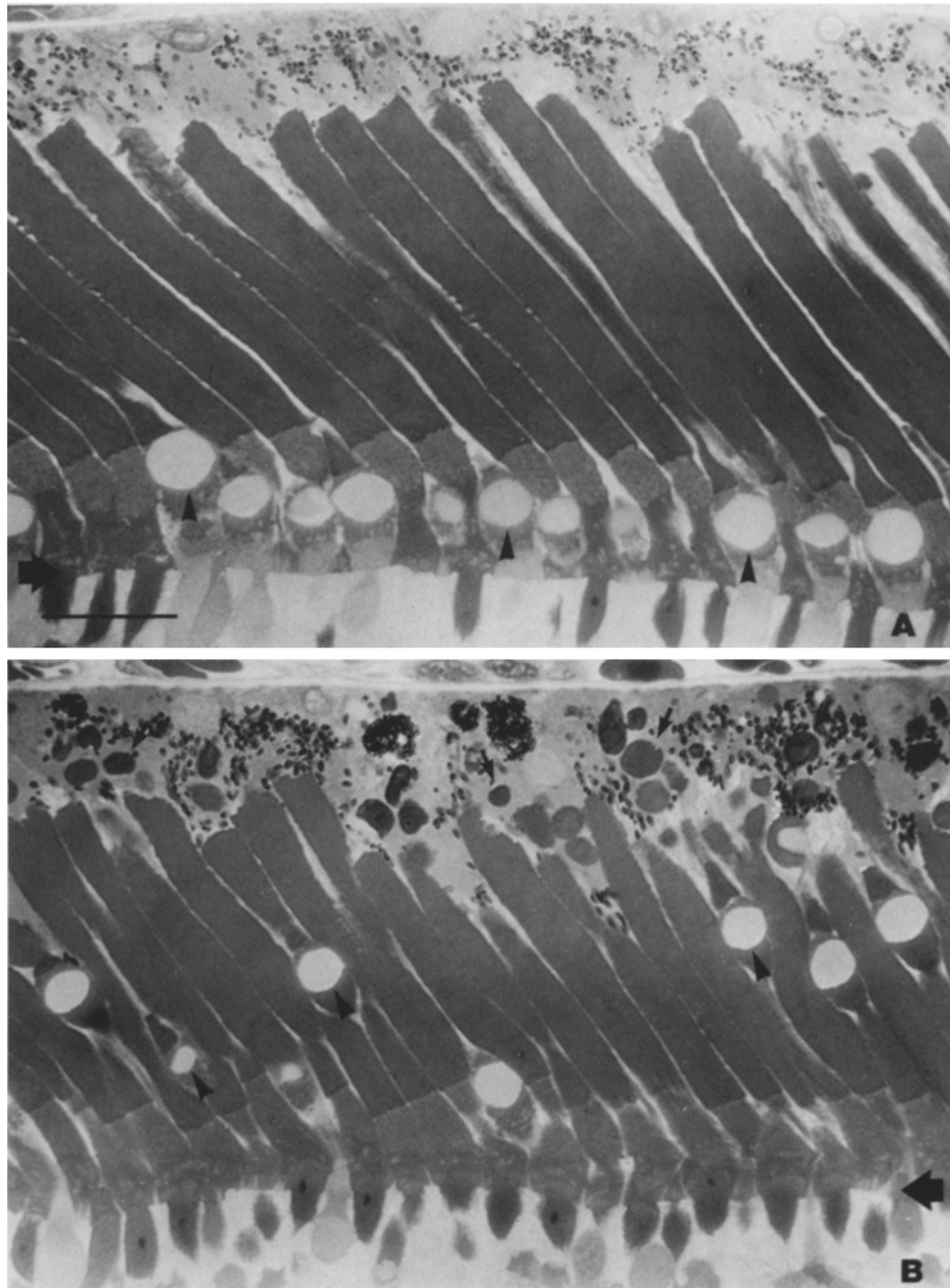
constant-light-treated eyecups can mimic darkness by promoting cone elongation in light. In addition, derivatives of cAMP reduce the number of photoreceptor-derived phagosomes found in pigment epithelial cells. The results are consistent with a role for cAMP in the regulation of both retinomotor movement and disc shedding.

MATERIALS AND METHODS

Postmetamorphic *X. laevis* (3.5–5.0 cm body length) obtained from Nasco, Inc., Fort Atkinson, WI, were maintained at 24–26°C under a lighting regime of 12L:12D and were fed commercial frog pellets (Nasco, Inc.) three times weekly. To study the light and dark processes responsible for cone elongation and disc shedding, eyecups were prepared according to procedures described previously (Besharse et al., 1980). Surgery was carried out in room light on animals that had been kept in constant light for 4 d. This “constant-light paradigm” was adopted because both cone elongation and shedding could be induced by dark treatment of the eyecups. Eyecups were placed in amphibian tissue culture medium (Grand Island Biological Co., Grand Island, NY) that had been supplemented with additional NaHCO_3 to a final concentration of 35 mM and gassed with 95% O_2 and 5% CO_2 ($\text{pH } 7.6 \pm 0.1$). Eyecups were kept in plastic culture dishes on a rotary shaker (60 rpm) in either light ($\sim 650 \text{ lm/m}^2$) or darkness for periods of 1–3 h. They were then fixed in a mixture containing 1% OsO_4 and 1.65% glutaraldehyde in 0.075 M cacodylate buffer ($\text{pH } 7.4$), dehydrated in ethanol, and embedded in Epon-Araldite or Spurr’s medium (Electron Microscopy Sciences, Fort Washington, PA) (Besharse et al., 1980).

Eyecups were treated with cyclic nucleotides, related purine compounds, and phosphodiesterase (PDE) inhibitors by addition of the drugs to the culture medium immediately before the beginning of experiments. The adenine compounds studied were cyclic adenosine 3',5'-monophosphate, adenosine hemisulfate, adenosine 5'-monophosphate, 8-bromoadenosine cyclic 3',5'-monophosphate, and $\text{N}^6, \text{O}^{2'}$ -dibutyryl-adenosine cyclic 3',5'-monophosphate (dbcAMP). We also studied the effects of $\text{N}^2, \text{O}^{2'}$ -dibutyryl-guanosine cyclic 3',5'-monophosphate (dbcGMP). All nucleosides and nucleotides were obtained from Sigma Chemical Co., St. Louis, MO, and their purity was verified by chromatography on cellulose plates (EM Laboratories, Inc., Elmsford, NY) or polyethyleneimine (PEI) cellulose plates (Schleicher and Scheuell, Inc., Keene, NH). The PEI thin-layer plates were developed with 0.75 M phosphoric acid (Cashel et al., 1969) or were developed sequentially with acetic acid, water, and 0.5 M LiCl (Bohme and Schultz, 1974). The cellulose plates were developed in a mixture containing five parts ethanol and two parts 0.5 M ammonium acetate (Posternak and Weiman, 1974). Spots were visualized under ultraviolet light. We also examined the effects of the phosphodiesterase inhibitors, 3-isobutylmethylxanthine, theophylline, and papaverine (Sigma Chemical Co.) either alone or in the presence of the dibutyryl derivatives of cAMP and cGMP.

Data on rod shedding and cone movement were obtained by light microscopic examination of 1- μm -thick sections through the dorso-ventral axis near the optic nerve. Sections were stained with Azure II (Sigma Chemical Co.). Phagosome counts were of those $>2 \mu\text{m}$ in their greatest dimension over the entire retinal expanse (2.5–4 mm) in a single section from each eyecup. Phagosomes were identified on the basis of stain intensity and shape as described previously (Besharse et al., 1977). The cone length in each eyecup was determined by measuring the distance between the external limiting membrane and the proximal edge of the oil droplet in a plane parallel to the long axis of the rod outer segments (see Fig. 1). 20 random measurements were made



per eyecup (10 each in dorsal and ventral retinal fields). Experimental treatments involved three to five eyecups (usually four), each from a different animal. Raw data were converted to an average value for each eyecup (i.e., phagosomes/mm retinal pigment epithelium [RPE] or average cone length). In some cases data are expressed as a percent of the maximum response. However, statistical treatment was carried out only on the raw data. Statistical evaluation of data involved analysis of variance, followed when appropriate by use of Duncan's new multiple range test for individual means (Scheffler, 1980; Edwards, 1968).

RESULTS

Disc Shedding and Cone Elongation In Vitro

Rod disc shedding in *X. laevis* maintained in cyclic light normally occurs within 2 h after light onset (Besharse et al., 1977), and a light-evoked shedding response of comparable magnitude can be elicited in eyecups kept in culture medium (Besharse et al., 1980). Disc shedding can also be studied effectively in constant-light-treated eyes where shedding is reduced to a very low level. Under these conditions shedding can be induced both in vivo (Currie et al., 1978) and in vitro (Besharse et al., 1980) by placing animals or eyecups in darkness. Incubation of eyecups from constant-light-treated animals in darkness for 3 h increases the phagosome content of RPE to a level more than two times that of comparable eyecups kept in light (Fig. 1, Table I). Occasionally, individual cones are seen in the process of shedding while in the extended position (see below), which suggests that the phagosome content of the RPE may represent a composite of phagosomes derived from both rods and cones. It is not possible in our material to distinguish rod from cone phagosomes. However, because of the large size of rod phagosomes and the small number of cones per unit of linear expanse of RPE, the shedding response measured is greatly dominated by rods. Light exposure of constant-light-treated animals or eyecups after an hour of darkness (Table I) results in an even greater enhancement of shedding.

Retinomotor movement of cones in *X. laevis* involves elongation of the inner segment myoid with extension of the outer segment outward toward the RPE in darkness (Fig. 1). Contraction occurs in light. Rod movement in *X. laevis* is difficult to quantify because total extensions are small. Pigment migration in

FIGURE 1. (*opposite*) Photomicrographs illustrating disc shedding and cone elongation in darkness. Eyecups from constant-light-treated animals were incubated for 3 h in light (A) or dark (B). Cones remain in the light-adapted (contracted) position in light but elongate substantially in darkness. Arrowheads indicate the proximal edge of the oil droplets and the large arrows indicate the level of the external limiting membrane. Rods are elongated in light and contracted in dark, but the total excursion is relatively small and difficult to quantify. Melanin granules do not exhibit measurable retinomotor movement in *X. laevis*. The large rod shedding response that occurs in darkness is reflected in the large phagosome content of the RPE (small arrows). Because a low level of shedding occurs even in light during a 3-h incubation, the field of view in A is unusual in that it contains no phagosomes. The calibration bar corresponds to 20 μ m.

the RPE does not occur (Saxén, 1954). In animals maintained in cyclic light, maximal cone extension occurs by the middle of the dark period, but contraction occurs before light onset (J.C. Besharse, unpublished data). Cone elongation also occurs on dark treatment of animals in constant light. In culture, cone elongation can be induced by dark treatment of eyecups from both constant-light- and cyclic-light-treated animals, although in the latter case dark treatment is effective only during the period of subjective night. In eyecups from constant-light animals we find that dark treatment at any time of day results in cone elongation comparable in magnitude to that occurring during the dark period in normal cyclic-light-treated animals (Fig. 1; Table I). Cone elongation under these conditions reaches a maximum within 1 h, and cones remain in the extended position during subsequent treatment for up to 3 h in darkness (Table I). In addition, returning eyecups to light after

TABLE I
EFFECTS OF LIGHT AND DARK ON DISC SHEDDING AND
RETINOMOTOR ELONGATION OF CONES IN EYECUPS FROM
CONSTANT-LIGHT-TREATED ANIMALS

Treat- ment	<i>n</i>	Phagosomes/ mm RPE*	Cone length* <i>μm</i>
Baseline‡	7	7.4±1.0	12.2±0.8
Light, 1 h	4	8.8±2.2	13.7±1.6
Dark, 1 h	4	11.8±2.6	30.5±4.8
Light, 3 h§	16	25.4±3.6	15.8±1.0
Dark, 3 h§	28	61.3±3.6	30.4±1.0
Dark, 1 h	8	78.1±5.9	16.4±1.7
Light, 2 h			

* Values are the mean plus or minus the standard error based on the number of eyecups (*n*) indicated. Where *n* > 4, the data were pooled from two to seven separate experiments.

‡ Eyecups were fixed at beginning of the incubation period.

§ Data are included in Tables II and III as control levels for observations on drug effects.

1 h of darkness results in contraction of cones to near their normal light-adapted position (Table I). The simultaneous occurrence of rod disc shedding and cone elongation in our in vitro system has made it possible to test the effects of cyclic nucleotides on those processes.

dbcAMP Promotes Cone Elongation

To determine whether treatments designed to increase cellular levels of cyclic nucleotides would mimic the effects of darkness on cone elongation and/or disc shedding, we incubated eyecups in light for 3 h in the presence or absence of cyclic nucleotides, PDE inhibitors, or a mixture of PDE inhibitor and cyclic nucleotide. We found that dbcAMP promoted cone elongation in light to an extent comparable to that occurring without drug treatment during 3 h of

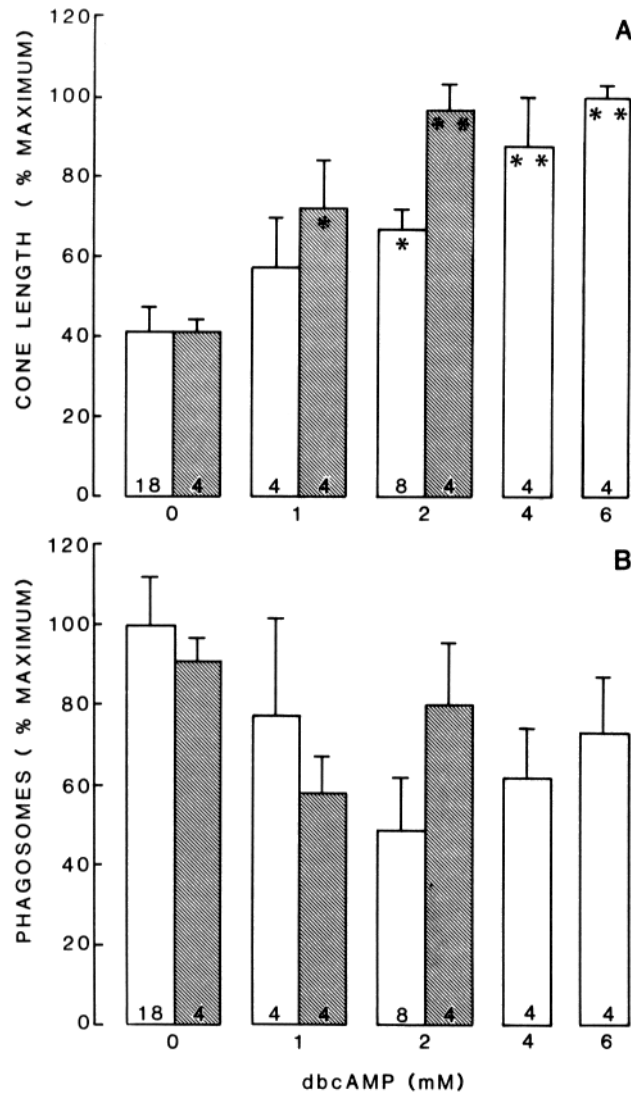


FIGURE 2. Histograms illustrating the effects of dbcAMP and papaverine on cone elongation (A) and disc shedding (B) in eyecups kept for 3 h in light. White columns represent dbcAMP concentration from 0 to 6 mM. Shaded columns represent groups that were treated with 0.1 mM papaverine at dbcAMP concentrations of 0, 1, and 2 mM. Raw data were converted to a ratio relative to their control group (no drug treatment) and are expressed in the histograms as a percent of maximum cone elongation or shedding. The maximum cone elongation recorded for a treatment group was $30.6 \pm 1.4 \mu\text{m}$. The average shedding value representing 100% was 26.1 ± 3.1 phagosomes/mm RPE. Asterisks indicate treatments that are significantly different from controls (* = $P \leq 0.05$; ** = $P \leq 0.001$).

darkness (Fig. 2A, Table II). As shown in Fig. 2A, cone length increased with concentration of dbcAMP, reaching a maximum at 4–6 mM, and the PDE inhibitor papaverine potentiated the effect of dbcAMP at lower concentration (1–2 mM). Isobutylmethylxanthine (IBMX) at 0.1 and 1 mM was similar to papaverine in that it had a negligible effect on cone elongation when provided alone, but increased the effect of dbcAMP by 30% when provided in combination with dbcAMP (Table II). cAMP and the 8-bromo derivative of cAMP also promoted cone elongation but were much less effective than dbcAMP; at 4 mM cones were, respectively, 18 and 34% longer than in controls.

TABLE II
EFFECTS OF CYCLIC NUCLEOTIDES, OTHER PURINE
COMPOUNDS, AND PHOSPHODIESTERASE INHIBITORS ON
ROD DISC SHEDDING AND CONE RETINOMOTOR
MOVEMENTS IN EYECUPS KEPT IN LIGHT FOR 3 H

Treatment	Concentration	<i>n</i>	Phagosomes/ mm RPE	Cone length
	<i>mM</i>			<i>μm</i>
Control*	—	16	25.4±3.6	15.8±1.0
dbcAMP‡	2	8	12.0±3.4§	25.5±1.7
dbcGMP‡	2	9	25.9±5.1	13.3±1.4
dbcGMP	4	4	23.8±5.4	12.4±0.6
IBMX	0.1	8	17.7±4.2	14.9±1.5
IBMX	1	4	10.2±1.3§	18.1±0.8
IBMX + dbcAMP**	0.1+2	4	9.5±4.7§	33.2±2.4¶
IBMX + dbcGMP**	0.1+2	4	17.6±3.7§	16.5±1.5
Adenosine	2	4	30.3±2.9	14.9±2.2
5'AMP	2	8	39.4±5.0	12.2±1.0

* Control data are as in Table I.

‡ The dibutyl derivative of cAMP or cGMP.

§ Values significantly different from controls; $P \leq 0.05$.

|| $P \leq 0.01$.

¶ $P \leq 0.005$.

** Both drugs were added together at 2 mM for the cyclic nucleotide and 0.1 mM for IBMX.

In contrast to the positive effect obtained with dbcAMP, cone elongation did not occur in the presence of adenosine or adenosine 5'-monophosphate (5'AMP). The slight reduction in cone length seen with 5'AMP by our criteria ($P = 0.05$) was not significant statistically. Furthermore, dbcGMP alone at 2 and 4 mM or in combination with IBMX had no effect (Table II). These results indicate that dbcAMP mimics darkness by promoting cone elongation and they suggest that the effect is specific for derivatives of cAMP. Rod myoid shortening also appeared to be promoted by dbcAMP; because rod excursions were much smaller and difficult to measure accurately, quantitative data are not presented.

dbcAMP Inhibits Shedding

In the presence of dbcAMP and PDE inhibitors, separately or in combination, the phagosome content of RPE was somewhat reduced compared with controls (Fig. 2B; Table II), which suggests a possible inhibitory effect on disc shedding and/or phagocytosis. Under the conditions of those experiments (incubation in light), however, levels of shedding were low even in controls and the inhibitory effects noted were quite variable. We therefore examined the effects of drugs on eyecups in darkness where extensive rod shedding and cone elongation occur without drug treatment (Table I). As shown in Fig. 3A, cones achieved near-maximal elongation in dark controls and further significant increases in cone length were not stimulated by dbcAMP alone or in the presence of papaverine. However, dbcAMP reduced the phagosome content of RPE to <45% of controls at 2–4 mM, and its effect was enhanced by papaverine (0.1 mM) at 1 and 2 mM (Fig. 3B). Although at the concentration used papaverine alone had no significant effect on shedding, we found that the methylxanthine PDE inhibitors, IBMX and theophylline, both inhibited disc shedding (Table III). IBMX was particularly potent, and its effect was not enhanced when provided in combination with dbcAMP or dbcGMP (Table III).

In an attempt to determine whether the effect on disc shedding was specific for dbcAMP, we found that both dbcGMP and adenosine had a small inhibitory effect on disc shedding that was not statistically significant ($P = 0.05$), and that 5'AMP had no measurable effect (Table III). None of the three treatments had a significant effect on cone length. Our data demonstrate that conditions that raise cyclic nucleotide levels will inhibit phagosome formation in RPE and suggest that cAMP may play a role in the regulation of shedding and phagocytosis.

IBMX inhibited cone elongation in darkness (Table III). This was in direct contrast to its effect in light (Table II), where it potentiated the stimulatory effect of dbcAMP on cone elongation. The inhibitory effect was detected when IBMX was used alone or in combination with dbcAMP and dbcGMP. Because neither dbcAMP nor dbcGMP influenced cone length in darkness when used alone, the inhibitory effect observed when coupled with IBMX can probably be attributed to IBMX. In contrast, theophylline alone was as effective as IBMX as an inhibitor of disc shedding but had no effect on cone elongation in darkness (Table III). Papaverine, when administered alone, had no effect on either process, but potentiated the effect of dbcAMP on both processes (Figs. 2A and 3B). Although we have not further analyzed the inhibitory effect of IBMX, our data suggest differential effectiveness of the PDE inhibitors on cone elongation and disc shedding. They also raise the possibility that the inhibitory effect of IBMX in darkness may not involve cAMP.

DISCUSSION

Each of the experiments reported in this paper was carried out on eyecups prepared from animals in which both disc shedding and cone elongation had

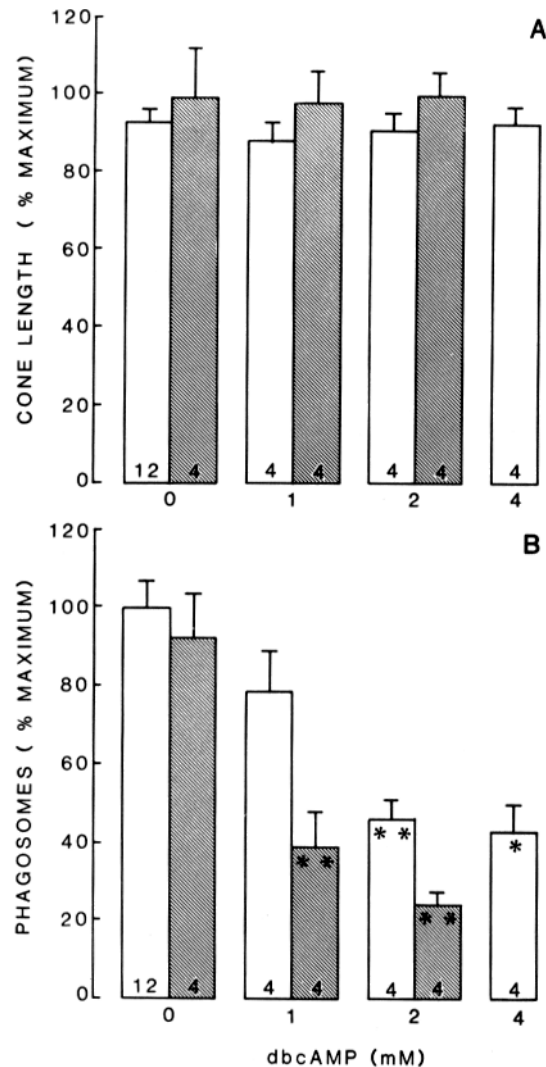


FIGURE 3. Histograms illustrating the effects of dbcAMP and papaverine on cone elongation (A) and disc shedding (B) in eyecups kept for 3 h in dark. White columns represent dbcAMP concentrations from 0 to 4 mM. Shaded columns represent groups that were treated with 0.1 mM papaverine at dbcAMP concentrations of 0, 1, and 2 mM. Raw data were converted to a ratio relative to their control group (no drug treatment) and are expressed in the histogram as percent of maximum cone elongation or shedding. The maximum cone elongation recorded for any group was $32.9 \pm 2.1 \mu\text{m}$. The average shedding value representing 100% was 72 ± 5.3 phagosomes/mm RPE. Asterisks indicate treatments that are significantly different from controls (* = $P \leq 0.05$; ** = $P \leq 0.001$).

been reduced by prior constant-light treatment. This approach was based on the previous demonstration that constant-light treatment greatly reduces disc shedding (Besharse et al., 1977) and that returning constant-light-treated animals to darkness initiates a shedding event involving most of the rods in the retina (Currie et al., 1978; Hollyfield and Basinger, 1978; Besharse et al., 1980; Besharse and Dunis, 1981). These observations emphasize the importance of a dark-dependent process in the control of disc shedding. Although rhythmic rod shedding in animals maintained on a cyclic photoperiod occurs as a peak soon after light onset, light itself is not an essential trigger for shedding in any of the species examined thus far. In the albino rat and in *X.*

TABLE III
EFFECTS OF CYCLIC NUCLEOTIDES, RELATED PURINE COMPOUNDS, AND PHOSPHODIESTERASE INHIBITORS ON DISC SHEDDING AND CONE RETINOMOTOR MOVEMENTS IN EYECUPS KEPT IN DARK FOR 3 H

Treatment	Concentration	<i>n</i>	Phagosomes/ mm RPE	Cone length
	<i>mM</i>			μm
Control*	—	28	61.3 \pm 3.6	30.4 \pm 1.0
dbcAMP‡	2	9	36.0 \pm 5.8§	30.1 \pm 1.2
dbcGMP‡	2	12	50.3 \pm 5.7	31.2 \pm 1.4
IBMX	0.1	4	21.4 \pm 2.0	19.6 \pm 5.2§
Theophylline	1	4	16.7 \pm 3.5§	33.5 \pm 0.5
IBMX + dbcAMP¶	0.1–2	4	24.2 \pm 5.3	17.1 \pm 1.3
IBMX + dbcGMP¶	0.1–2	4	27.6 \pm 10.2**	17.4 \pm 2.1
Adenosine	2	8	54.6 \pm 9.6	30.6 \pm 0.9
5'AMP	2	8	65.2 \pm 6.5	28.5 \pm 1.0

* Control data are as in Table I.

‡ The dibutyryl derivative of cAMP or cGMP.

§ Values significantly different from controls; $P \leq 0.01$.

|| $P \leq 0.005$.

¶ Both drugs added together at 2 mM for the cyclic nucleotide and 0.1 mM for IBMX.

** $P \leq 0.05$.

laevis, disc shedding free-runs as an endogenous, circadian rhythm in darkness but is blocked in constant light (Besharse et al., 1977; LaVail, 1976, 1980; Goldman, et al., 1980). Continued shedding also occurs in darkness in pigmented mice (Besharse and Hollyfield, 1979). Although a free-running rhythm of disc shedding has not been detected in *Rana pipiens* (Basinger et al., 1976), spontaneous shedding occurs after several days of darkness (Hollyfield et al., 1980), and the shedding rhythm can be quickly entrained to a 24-h cycle of alternating high and low temperature in darkness (Basinger and Hollyfield, 1980). The general requirement for darkness before a rod-shedding event and

the relatively low level of rod shedding during the dark period of the normal circadian cycle raise a question as to what factors prevent nighttime shedding in cyclic-light animals. A possible explanation is that an inhibitory process in darkness prevents shedding and that release of the inhibitory process at light onset or by a circadian oscillator leads to a synchronous burst of shedding. The data presented in this paper suggest that such an inhibitory process may be mediated by cAMP.

Retinomotor cone movement in *X. laevis* is extensive, but rod movement and melanin pigment migration are poorly developed (Saxén, 1954). Several of our unpublished observations suggest that cone position in *X. laevis* is influenced by a circadian oscillator, as is true for a variety of teleost species (see Levinson and Burnside, 1981). For example, dark-induced cone elongation does not occur in intact animals or in eyecups during subjective day but will occur after the time of normal light offset. In addition, cone contraction to near the light-adapted position occurs before light onset in cyclic-light-maintained animals. In this paper we demonstrate that cone myoids elongate in darkness and contract in light in eyecups from constant-light-treated animals. This has made it possible for us to study cone movement in an in vitro preparation that also sustains disc shedding. It should be emphasized, however, that the rod shedding response observed in this study is different from diurnal shedding in that it occurs in darkness in parallel with cone elongation.

The principal conclusions of this study are that treatments that elevate intracellular concentrations of cAMP promote retinomotor cone elongation and decrease phagosome levels in the pigment epithelium. The effect on cones occurs in light, is comparable in magnitude to that seen in darkness without drug treatment, and is specific in that dbcGMP, adenosine, and 5'AMP are without effect. Similar effects on cone elongation have been observed in fishes after intraocular injection and culture with cyclic nucleotides and PDE inhibitors (Burnside et al., 1982). The results of both studies strongly suggest that cAMP is a key intermediate in cellular reactions controlling photoperiod-related movements in rods, cones, and pigment epithelium and that elevation of cAMP triggers dark-adaptive movements. This hypothesis implies that cAMP levels within the retina or the photoreceptor-RPE complex should vary within the light-dark cycle. Several lines of evidence suggest that such changes occur. Retinal cAMP levels have been reported to be higher in darkness than in light in rabbits (Orr et al., 1976), mice (DeVries et al., 1978), and green sunfish (Burnside et al., 1982), but data on the cone-dominated retinas of ground squirrels are at variance in that retinal cAMP has been reported to be unaltered (DeVries et al., 1979) or greatly reduced (Farber et al., 1981) in light. Although much of the light-labile cAMP in rod-dominated retina of rabbits is found in the outer plexiform layer (Orr et al., 1976), there are also significant light-dark differences in cAMP within other parts of the retina including photoreceptor inner segments (Orr et al., 1976). We have recently obtained data indicating that cAMP levels in *X. laevis* retinas are altered by photoperiod in both intact animals and cultured eyecups (K. Dutt and J. C. Besharse, manuscript in preparation). Retinomotor movements and disc

shedding are also known to be influenced by an endogenous circadian rhythm (Levinson and Burnside, 1981; Besharse et al., 1977; LaVail, 1980). The nature of the rhythmic factor has not been elucidated, but it could also act by modulating cytoplasmic levels of cAMP. This is consistent with the observation of circadian fluctuations of cAMP levels in mice (DeVries et al., 1978).

Photoreceptor disc shedding appears to be related to retinomotor movements in lower vertebrates in the sense that disc detachment and phagocytosis normally occur while each photoreceptor type is in the extended position with its apical tip in optimal proximity to the cell body of an RPE cell. Thus, rod photoreceptor shedding occurs after light onset when rods are extended (Besharse et al., 1977) and cone shedding occurs after light offset when cones are extended (Young, 1977). These observations raise the possibility that retinomotor movements and disc shedding may be mechanically coupled processes, modulated at least in part by the same cyclic nucleotide system. Our observation of rod shedding in darkness while rods are contracted indicates that in *X. laevis* the normal relationship of rod retinomotor movement and shedding can be uncoupled, and suggests that in this system the effect of dbcAMP on shedding may be mediated by a mechanism independent of rod movement. It should be emphasized, however, that we have not obtained quantitative data on rod movement because in *X. laevis* the total excursions are quite small. Further analysis of the potential relationship of retinomotor movement and shedding can probably be carried out most effectively in a species that exhibits greater total excursions of each photoreceptor type.

The inhibition of rod disc shedding reported here is similar to observations on monolayer cultures of RPE in which cAMP and PDE inhibitors reduced phagocytosis of isolated outer segments (Edwards and Bakshian, 1980). In the latter study, however, the effect was not specific; a series of related purine compounds including dbcGMP, adenosine, adenine, and guanosine all had significant inhibitory effects. Although the cultured RPE system has the virtue that the drug effects can be attributed to a single cell type, the results so far are equivocal with regard to a role for cAMP in phagocytosis of rod outer segment discs. In contrast, our data suggest a specific role for cAMP in the control of disc shedding but are equivocal with regard to the cellular site of the effect. Because our assay of disc shedding requires the controlled interaction of both RPE and photoreceptors and reflects an endpoint dependent on that interaction, we are unable to discriminate between effects on photoreceptors or RPE. Furthermore, it remains possible that drug effects on diverse mechanisms in the two cell types could result in a similar effect on phagosome levels in RPE. This problem will only be resolved when the separate roles of photoreceptors and RPE in shedding are more fully understood.

Although precise mechanisms are not known, it is possible that cAMP acts within the photoreceptor-RPE complex by regulating the phosphorylation of endogenous proteins. It seems likely that given the diverse processes affected by cAMP (i.e., cone elongation, rod contraction, shedding) different protein systems would be involved. Because cone elongation appears to be mediated by a system of cytoplasmic microtubules (Warren and Burnside, 1978), protein

phosphorylation may modulate microtubule assembly-disassembly or microtubule sliding (Dedman et al., 1979). An alternative mechanism would involve inactivation of the actin system responsible for cone contraction (Burnside, 1978). As noted above, disc shedding and retinomotor movements may be mechanically coupled so that phosphorylation-dephosphorylation cycles of the same endogenous proteins in rods and cones could play a role in the regulation of both retinomotor movement and shedding. However, it is also possible that the RPE plays a major role in the control of shedding. In this regard, it is particularly interesting that cAMP (and other purine compounds) will inhibit phagocytosis of rod outer segments by cultured RPE cells (Edwards and Bakshian, 1980). Furthermore, several laboratories have shown that in polymorphonuclear leukocytes, a cell in which phagocytosis is regulated by exogenous agonists, cAMP reduces both phagocytosis and lysosomal enzyme secretion (Ignasso and Cech, 1976; Smith, 1977; Stossel et al., 1972). Our data suggest that further analysis of the role of cAMP in the photoreceptor-RPE complex may be useful in defining the cellular mechanisms of both photoreceptor motility and disc shedding.

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REFERENCES

- AREY, L. B. 1915. The occurrence and the significance of photomechanical changes in the vertebrate retina—an historical survey. *J. Comp. Neurol.* **25**:535–554.
- BASINGER, S., R. HOFFMAN, and M. MATTHES. 1976. Photoreceptor shedding is initiated by light in the frog retina. *Science (Wash. D. C.)*. **194**:1074–1076.
- BASINGER, S. F., and J. G. HOLLYFIELD. 1980. Control of rod shedding in the frog retina. *Neurochemistry*. **1**:81–92.
- BESHARSE, J. C., and D. A. DUNIS. 1982. Rod photoreceptor disc shedding *in vitro*: inhibition by cytochalasins and activation by colchicine. In *Structure of the Eye*. J. G. Hollyfield, editor. Elsevier North-Holland, Amsterdam. 85–96.
- BESHARSE, J. C., and J. G. HOLLYFIELD. 1979. Turnover of mouse photoreceptor outer segments in constant light and darkness. *Invest. Ophthalmol. Vis. Sci.* **18**:1019–1024.
- BESHARSE, J. C., J. G. HOLLYFIELD, and M. I. RAYBORN. 1977. Turnover of rod photoreceptor outer segments. II. Membrane addition and loss in relationship to light. *J. Cell Biol.* **75**:507–527.
- BESHARSE, J. C., R. O. TERRILL, and D. A. DUNIS. 1980. Light-evoked disc shedding by rod photoreceptors *in vitro*: relationship to medium bicarbonate concentration. *Invest. Ophthalmol. Vis. Sci.* **19**:1512–1517.
- BOHME, E., and G. SCHULTZ. 1974. Separation of cyclic nucleotides by thin layer chromatography on polyethyleneimine cellulose. *Methods Enzymol.* **68**:27–38.

- BURNSIDE, B. 1978. Thin (actin) and thick (myosin-like) filaments in cone contraction in the teleost retina. *J. Cell Biol.* **78**:227-246.
- BURNSIDE, B., R. T. FLETCHER, G. J. CHADER, and M. EVANS. 1982. Induction of dark-adaptive retinomotor movement (cell elongation) in teleost retinal cones by cyclic adenosine 3',5'-monophosphate. *J. Gen. Physiol.* **79**:759-774.
- CASHEL, M., R. A. LAZZARINI, and B. KALBACHER. 1969. An improved method for thin-layer chromatography of nucleotide mixtures containing ^{32}P -labeled orthophosphate. *J. Chromatog.* **40**:103-109.
- CURRIE, J. R., J. G. HOLLYFIELD, and M. E. RAYBORN. 1978. Rod outer segments elongate in constant light: darkness is required for normal shedding. *Vision Res.* **18**:995-1003.
- DEDMAN, J. R., B. R. BRINKLEY, and A. R. MEANS. 1979. Regulation of microfilaments and microtubules by calcium and cyclic AMP. *Adv. Cyclic Nucleotide Res.* **11**:131-174.
- DEVRIES, G. W., A. I. COHEN, I. A. HALL, and J. A. FERRENDELLI. 1978. Cyclic nucleotide levels in normal and biologically fractionated mouse retina: effects of light and dark-adaptation. *J. Neurochem.* **31**:1345-1351.
- DEVRIES, G. W., A. I. COHEN, O. H. LOWRY, and J. A. FERRENDELLI. 1979. Cyclic nucleotides in the cone-dominated ground squirrel retina. *Exp. Eye Res.* **29**:315-321.
- EDWARDS, A. L. 1968. *Experimental Design in Psychological Research*. 3rd edition. Holt-Rinehart and Winston, Inc., New York. 455 pp.
- EDWARDS, R. B., and S. BAKSHIAN. 1980. Phagocytosis of outer segments by cultured rat pigment epithelium reduction by cAMP and phosphodiesterase inhibitors. *Invest. Ophthalmol. Vis. Sci.* **19**:1184-1188.
- FARBER, D. B., D. W. SOUZA, D. G. CHASE, and R. W. LOLLEY. 1981. Cyclic nucleotides of cone-dominant retinas: reduction of cyclic AMP levels by light and by cone degeneration. *Invest. Ophthalmol. Vis. Sci.* **20**:24-31.
- FLETCHER, R. T., and G. J. CHADER. 1976. Cyclic GMP: control of concentration by light in photoreceptors. *Biochem. Biophys. Res. Commun.* **70**:1297-1302.
- FUNG, B. K. K., J. B. HURLEY, and L. STRYER. 1981. Flow of information in the light-triggered cyclic nucleotide cascade of vision. *Proc. Natl. Acad. Sci. U.S.A.* **78**:152-156.
- GOLDMAN, A. I., P. S. TIERSTEIN, and P. J. O'BRIEN. 1980. The role of ambient lighting in circadian disc shedding in the rod outer segment of the rat retina. *Invest. Ophthalmol. Vis. Sci.* **19**:1257-1267.
- GORIDIS, C., P. F. URBAN, and P. MANDEL. 1977. The effect of flash illumination on the endogenous cyclic GMP content of isolated frog retinae. *Exp. Eye Res.* **24**:171-177.
- HOLLYFIELD, J. G., and S. F. BASINGER. 1978. Photoreceptor shedding can be initiated within the eye. *Nature (Lond.)*. **274**:794.
- HOLLYFIELD, J. G., J. C. BESHARSE, and M. E. RAYBORN. 1976. The effect of light on the quantity of phagosomes in the pigment epithelium. *Exp. Eye Res.* **23**:623-635.
- HOLLYFIELD, J. G., M. E. RAYBORN, and S. F. BASINGER. 1980. Membrane turnover in rod outer segments: changing patterns of shedding after extended periods of constant light and darkness. In *The Effects of Constant Light on Visual Processes*. T. P. Williams and B. N. Baker, editors. Plenum Press, New York. 389-399.
- HUBBELL, W. L., and M. D. BOWNS. 1979. Visual transduction in vertebrate photoreceptors. *Annu. Rev. Neurosci.* **2**:17-34.
- IGNASSO, L. J., and S. Y. CECI. 1976. Bidirectional regulation of lysosomal enzyme secretion and phagocytosis in human neutrophils by guanosine 3',5'-monophosphate and adenosine 3',5'-monophosphate. *Proc. Soc. Exp. Biol. Med.* **151**:448-452.

- KILBRIDE, P., and T. G. EBREY. 1979. Light initiated changes of cyclic guanosine monophosphate levels in the frog retina measured with quick-freezing techniques. *J. Gen. Physiol.* **74**:415-426.
- LAVAIL, M. M. 1976. Rod outer segment disc shedding in rat retina: relationship to cyclic lighting. *Science (Wash. D. C.)*. **194**:1071-1074.
- LAVAIL, M. M. 1980. Circadian nature of rod outer segment disc shedding in the rat. *Invest. Ophthalmol. Vis. Sci.* **19**:407-411.
- LEVINSON, G., and B. BURNSIDE. 1981. Circadian rhythms in teleost retinomotor movements. A comparison of the effects of circadian rhythm and light condition on cone length. *Invest. Ophthalmol. Vis. Sci.* **20**:294-303.
- ORR, H. T., O. H. LOWRY, A. I. COHEN, and J. A. FERRENDELLI. 1976. Distribution of 3':5'-cyclic AMP and 3':5'-cyclic GMP in rabbit retina *in vivo*: selective effects of dark and light adaptation and ischemia. *Proc. Natl. Acad. Sci. U. S. A.* **73**:4442-4445.
- POSTERNAK, T., and G. WEIMAN. 1974. The preparation of acylated derivatives of cyclic nucleotides. *Methods Enzymol.* **68**:399-409.
- SAXÉN, L. 1954. The development of the visual cells. Embryological and physiological investigations on amphibia. *Ann. Acad. Sci. Fenn. (Ser. A)*. **23**:1-107.
- SCHEFLER, W. C. 1980. Statistics for the Biological Sciences. 2nd edition. Addison-Wesley Publishing Co., Reading, Mass. 230 pp.
- SMITH, R. J. 1977. Phagocytic release of lysosomal enzymes from guinea pig neutrophils—regulation by corticosteroids, autonomic neurohormones and cyclic nucleotides. *Biochem. Pharmacol.* **26**:2001-1009.
- STOSSEL, T. P., R. J. MASSON, J. HARTWIG, and M. VAUGHAN. 1972. Quantitative studies of phagocytosis by polymorphonuclear leukocytes: use of emulsions to measure initial rate of phagocytosis. *J. Clin. Invest.* **51**:615-624.
- WARREN, R. H., and B. BURNSIDE. 1978. Microtubules in cone myoid elongation in the teleost retina. *J. Cell Biol.* **78**:247-259.
- WHEELER, G. L., and M. W. BITENSKY. 1977. A light-activated GTPase in vertebrate photoreceptors: regulation of light-activated cyclic GMP phosphodiesterase. *Proc. Natl. Acad. Sci. U. S. A.* **74**:4238-4242.
- WOODRUFF, M. L., D. BOWND, S. H. GREEN, J. L. MORRISEY, and A. SHEDLOVSKY. 1977. Guanosine 3',5'-cyclic monophosphate and the *in vitro* physiology of frog photoreceptor membranes. *J. Gen. Physiol.* **69**:667-679.
- YOUNG, R. W. 1977. The daily rhythm of shedding and degradation of cone outer segment membranes in the lizard retina. *J. Ultrastruct. Res.* **61**:172-185.
- YEE, R., and P. A. LIEBMAN. 1978. Light-activated phosphodiesterase of the rod outer segment kinetics and parameters of activation and deactivation. *J. Biol. Chem.* **253**:8902-8909.